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DOCUMENTATION OF THE CHROMOSOME NUMBER FOR THE CALIFORNIA ENDEMIC, *TOXICOSCORDION EXALTATUM* (LILIALES: MELANTHIACEAE)

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Abstract

The mitotic chromosome count for *Toxicoscordion exaltatum* ($2n = 22$), documented for the first time here, matches the number formerly reported for other species in the genus. A base number of $x = 11$ is synapomorphic for *Toxicoscordion*. An updated overview of this recently resurrected segregate genus is given.

Key Words: California endemic, chromosome number, Melanthiaceae, *Toxicoscordion*, *Zigadenus*.

Tribe Melanthieae (Liliales: Melanthiaceae) comprises seven genera (ca. 65–95 spp.) of predominately woodland and/or alpine perennial herbs occurring mainly in the temperate to Arctic zones of the Northern Hemisphere: *Amianthium* A. Gray (1 sp.), *Anticlea* Kunth (9–11 spp.), *Schoenocaulon* A. Gray (26 spp.), *Stenanthium* (A. Gray) Kunth (3 spp.), *Toxicoscordion* Rydb. (8 spp.), *Veratrum* L. *s.l.* (17–45 spp.), and *Zigadenus* Michx. *s.s.* (1 sp.). These generic circumscriptions (most novel) are supported by parsimony analyses of *trnL-F* (plastid) DNA and ITS (nuclear ribosomal) sequence data (Zomlefer et al. 2001, 2003, 2006a, b). A significant consequence of the molecular studies was the reassessment of the traditional *Zigadenus s.l.*, a poorly defined assemblage with a complex taxonomic history involving several segregate genera (summaries in Zomlefer 1997; Zomlefer et al. 2006a). Contemporary treatments (e.g., Schwartz 2002) have typically accepted the monotypic segregate *Amianthium* with the remaining ca. 20 species maintained in *Zigadenus s.l.* Based on these molecular data, however, *Zigadenus s.l.* is polyphyletic and forms five strongly supported clades correlating with certain geographical distribution, morphological characters, and chromosome number.

One well-defined clade corresponds to *Toxicoscordion*, originally described by Rydberg (1903), and sometimes recognized as *Zigadenus* sect. *Chitonia* (Salisb.) Baker (Preece 1956; Schwartz 1994). This *Zigadenus* segregate is defined by the morphological synapomorphies of conspicuously clawed tepals (especially the inner three) and one obovate gland per tepal (Fig. 1B; Zomlefer et al. 2001; Zomlefer and Judd 2002). *Toxicoscordion* comprises ca. eight species (Table 1) restricted to the midwestern United States to western North America (Fig. 1A) and includes the widely distributed poisonous "death camas" plants of the rangelands such as *T. nuttallii* (A. Gray) Rydb., *T. paniculatum* (Nutt.) Rydb., and *T. venenosum* (S. Watson) Rydb. (see Marsh et al. 1915, 1926).

However, the focal taxon of this paper, *T. exaltatum* (Eastw.) A. Heller (giant death camas), has a more limited distribution: mid-elevations (1000 to 1800 m) along margins of mixed coniferous forest on the western slopes of the Sierra Nevada Mountains in California (Fig. 1A; Walsh 1940; Preece 1956; Schwartz 1994, 2002). (Reports of this taxon from the foothills of the Sierra Nevada near Chinese Camp, Tuolumne Co. [see Preece 1956] are based on several collections of *T. fremontii*, a species otherwise known only from the Coast Ranges in California [McNeal and Zomlefer 2009].) *Toxicoscordion exaltatum* is the largest species in the genus with relatively large bulbs (6–10 cm long; 3–5 cm in diam.) and long flowering stems to 100 cm tall (McNeal 1993). These robust plants often occur in large populations of hundreds to thousands of individuals—creating a remarkable display (McNeal personal observation).

The chromosome number $2n = 22$ (or $n = 11$) has been verified for all species of *Toxicoscordion* (Table 1; Fig. 2; see Zomlefer 2003), except for *T. exaltatum* with an undocumented citation of $n = 11$ by McNeal (1993; Schwartz 2002). Since chromosome number is a significant and likely an invariable apomorphy for genera of tribe Melanthieae (e.g., Zomlefer and Smith 2002;

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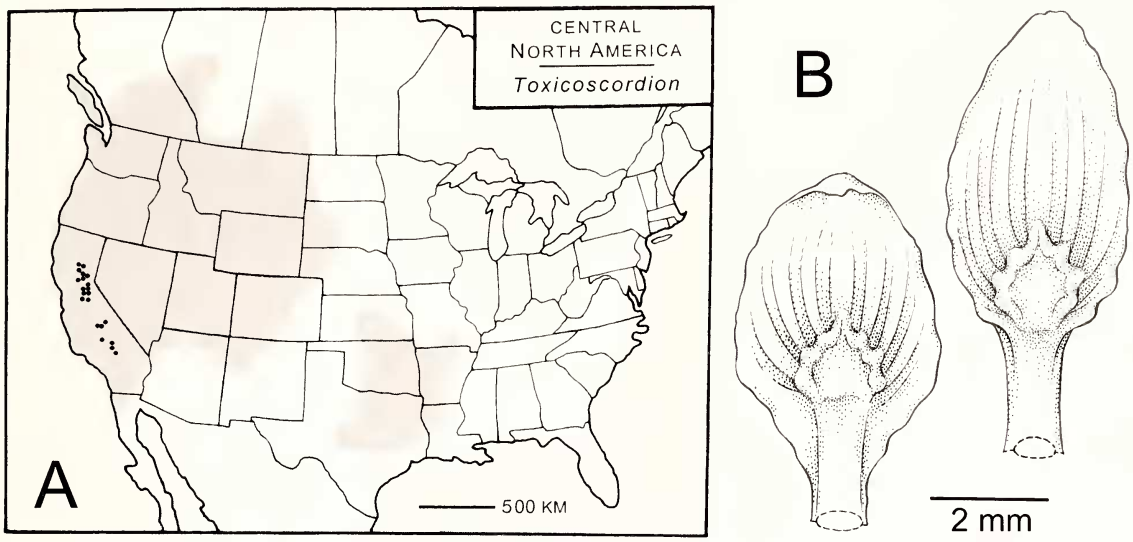

FIG. 1. Distinctive characteristics of *Toxicoscordion*. A. General distribution map of *Toxicoscordion* (gray shading) with dots indicating localities of the California endemic *T. exaltatum*. (The south-midwestern disjunct range of the genus comprises *T. nuttallii*.) Data from Walsh (1940), Preece (1956), and Schwartz (1994, 2002). B. Adaxial surface of an outer (left) and an inner (right) tepal of *T. exaltatum* showing the generic autapomorphies: claw plus a single obovate gland. Dashed ellipse = filament insertion. The claws of the inner tepals are typically more pronounced than those of the outer whorl. Drawn from live material (*McNeal & Smith 4749*; CPH, GA) by W. B. Zomlefer.

Zomlefer 2003), 22 is the predicted mitotic count for all *Zigadenus* species now transferred to *Toxicoscordion*. We here document the chromosome number of *T. exaltatum*.

MATERIALS AND METHODS

Several bulbs of *Toxicoscordion exaltatum* were collected during field work by the first author on 12 May 2006, stored in paper bags for three months, and then placed on moist peat moss in a growth chamber at 4°C with 16 hr of light and 8 hr of darkness per day. Growing root tips (1–2 cm long) were collected at ca 7:00 a.m. and soaked in 0.2% colchicine for 26–28 hr, rinsed in deionized water, and fixed in Carnoy's solution (3 ethanol: 1 acetic acid). Root tips thus prepared were stored in the freezer for at least 24 hr, transferred to 70% ethanol to slow tissue hardening, and returned to the freezer. Squash

TABLE 1. THE SPECIES OF *TOXICOSCORDION* AND THE ORIGINAL DOCUMENTATION OF THE MITOTIC (2*n*) AND/OR MEIOTIC (*n*) CHROMOSOME NUMBERS. See also Zomlefer (2003).

Taxon	Chromosome number		Original source(s)
	n	2*n*	
T. brevibracteatus (M. E. Jones) R. R. Gates	11	—	Lewis (1959); Cave (1970)
T. exaltatum (Eastw.) A. Heller	11	—	this paper
T. fontanum (Eastw.) Zomlefer & Judd	11	—	Preece (1956, as *Zigadenus venenosus* var. *fontanus*); Cave (1970)
T. fremontii (Torr.) Rydb.	11	—	Miller (1930); Preece (1956, as *Zigadenus fremontii* var. *fremontii*); Cave (1970, as *Z. fremontii* var. *fremontii* and var. *inezianus*)
T. micranthum (Eastw.) A. Heller	11	—	Preece (1956, as *Zigadenus venenosus* var. *micranthus*)
T. nuttallii (A. Gray) Rydb.	—	22	Zomlefer (2003)
T. paniculatum (Nutt.) Rydb.	11	22	Preece (1956)
T. venenosum (S. Watson) Rydb.	11	22	Preece (1956, as *Zigadenus venenosus* var. *gramineus* and var. *venenosus*); Cave (1970); Taylor & Taylor (1977, as *Z. venenosus* var. *gramineus*); Hartman & Crawford (1971, as *Z. venenosus* var. *gramineus*)

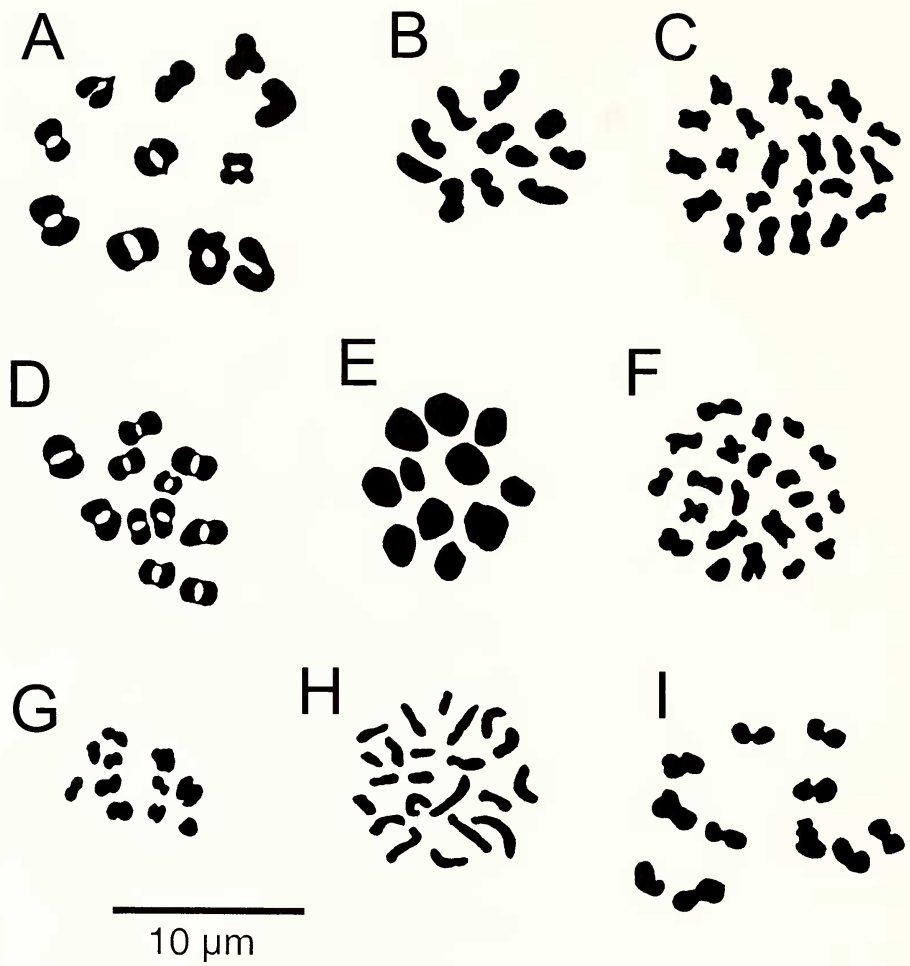

FIG. 2. Summary illustration of previously documented meiotic (n = 11) and/or mitotic ($2n$ = 22) chromosomes of *Toxicoscordion* (A–B, D–I) and the new report for *T. exaltatum* (C: $2n$ = 22). A. *T. brevibracteatus*, diakinesis (n = 11). B. *T. fontanum*, anaphase II (n = 11). C. *T. exaltatum*, metaphase ($2n$ = 22), (*McNeal & Smith s.n.* [12 May 2006], CPH). D. *T. fremontii*, metaphase I (n = 11). E. *T. micranthum*, metaphase I (n = 11). F. *T. nuttallii*, metaphase ($2n$ = 22). G. *T. paniculatum*, metaphase ($2n$ = 22). H. *T. paniculatum*, metaphase I (n = 11). I. *T. venenosum*, metaphase I (n = 11). A, B, and I modified from Cave (1970); D, E, G, and H modified from Preece (1956); F from Zomlefer (2003).

preparations were made within 30–60 days according to protocols outlined by Brooks et al. (1963). Following fixation, roots were rinsed several times in deionized water and hydrolyzed in 1.0 N HCL at 60°C for 5 min and rinsed several times again. Root tips were trimmed to ca. 3 mm and placed on slides in saturated iron aceto-carmine and macerated with fine-tipped forceps. After application of a cover slip, the preparation was squashed using thumb pressure. Slides were mounted with euparal for future reference. Well-spread metaphase chromosomes were traced by the second author under a Leica DMLB Research Microscope with a camera lucida attachment. The herbarium voucher specimen, *McNeal & Smith s.n.* (12 May 2006), is deposited at CPH.

RESULTS AND DISCUSSION

The mitotic chromosome number of $2n$ = 22 for *Toxicoscordion exaltatum* is confirmed with plants from a population in El Dorado Co., California (Fig. 2C; Table 1), and the previous report by McNeal (1993) is now documented with a voucher specimen. The validation of the diploid number for *T. exaltatum* strengthens support for the monophyly of *Toxicoscordion*, as well as the phylogenetic significance of chromosome numbers as generic synapomorphies for tribe Melanthieae.

A probable base chromosome number of x = 8 is often cited for the tribe (summary in Zomlefer et al. 2006a). The diploid numbers of 22 for *Toxicoscordion* (Zomlefer 2003) and 20 for

Stenanthium (Zomlefer and Judd 2002) are exceptions to the multiples of eight prevalent in the other genera (Sen 1975; Tamura 1995; Lowry et al. 1987; Zomlefer 1997): *Amianthium* ($2n$ = 32), *Anticlea* ($2n$ = 32), *Schoenocaulon* ($2n$ = 16), and *Veratrum* (including *Melanthium*; $2n$ = 16, 32, 64, 80, 96). Due to the small size of the chromosomes of this tribe (ca. 2.0–4.0 μm in length), the few detailed karyological studies (e.g., Lee 1985) lack the detail to infer mechanisms of chromosomal evolution, although these chromosome numbers indicate the prevalence of polyploidy and/or aneuploid variation of the prospective basic number.

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